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## Rainfall and crop residue effects on soil dispersion and *Beauveria bassiana* spread to corn

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### Abstract

The entomopathogenic fungus *Beauveria bassiana* infects a wide range of insects and survives as a soil saprophyte and a plant endophyte. The objective of our study was to determine the role of rainfall in dispersing *B. bassiana* (Balsamo) Vuillemin to the surface of corn (*Zea mays*) from soil with different levels of crop residue. Laboratory studies which simulated field tillage systems, had levels of crop residue which covered 0.53 (control), 34, 59 and 84% of the soil surface. Simulated rainfall in a raindrop tower at an intensity of 73 mm/h caused a significant increase in the mean number of *B. bassiana* colony forming units (CFU) isolated from the surface of corn plants. Plants receiving rain had a mean ( $\pm$ S.E.M.) of  $8.8 \pm 2.8$  CFU per plant; controls had a mean of  $0.03 \pm 0.01$  CFU per plant. The mg of soil collected from the surface of plants also was significantly influenced by rainfall. Plants receiving rain had a mean of  $15.7 \pm 1.7$  mg of soil per plant while controls had a mean of  $3.4 \pm 0.4$  mg of soil per plant. Linear regression revealed highly significant negative relationships between the mean mg of soil and the mean number of CFU per plant, over the four levels of crop residue. The amount of soil and number of CFU per plant decreased significantly with increasing levels of crop residue. In field studies with conservation- and no-till systems, results were similar to those recorded in the raindrop tower. The mean number of CFU and mg of soil per plant were both higher in conservation-till plots than in no-till plots, where surface residue averaged 45 and 95%, respectively. Rainfall plays an active role in the dispersal of *B. bassiana* from the soil environment to the surface of whorl-stage corn. Increased levels of crop residue reduce the amount of soil and fungal transfer to the surface of young corn. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** *Beauveria bassiana*; Fungal dynamics; Rainfall; Crop residue; Epizootiology

### 1. Introduction

The effects of environmental factors on the dispersal of fungal plant pathogens has been, and continues to be, an important area of research. The factors most commonly studied are wind patterns for dispersal of dry-dispersed fungal spores and rainfall intensity,

duration, and droplet size for dispersal of soil-borne spores (Aylor, 1990; Madden, 1997). Rainfall is important in dispersal of fungal and plant pathogens (Faulwetter, 1917a,b). In the absence of insects that can act as vectors of fungal spores, rain splash is critical to fungal spore transport (Gregory et al., 1959; Hunter and Kunitomo, 1974; Schub, 1983). However, the influence of these environmental factors on the spread of entomogenous fungi, for the most part, is unknown.

*Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) is a ubiquitous, entomopathogenic fungus, virulent against European corn

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borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), that has been used extensively to suppress larval populations (Bartlett and Lefebvre, 1934; Beall et al., 1934; Stirrett et al., 1937; York, 1958; Riba, 1984; Lewis and Cossentine, 1986; Marcandier and Riba, 1986; Feng et al., 1988; Lewis and Bing, 1991). In addition to suppressing corn borer populations, *B. bassiana* forms an endophytic relationship with corn (Bing and Lewis, 1991, 1992). Endophytic *B. bassiana* also infects *O. nubilalis* larvae tunneling within the corn plant (Bing and Lewis, 1991). A conidium on the surface of a corn leaf germinates, the germ tube elongates, and penetrates the leaf cuticle just as it penetrates the cuticle of an insect (Wagner and Lewis, 2000). Conidia germinate regardless of topographical signals on the leaf surface, and hyphae penetrate the plant cuticle directly; microscopic observations suggest both an enzymatic and mechanical mechanism of penetration (Wagner and Lewis, 2000). Once in the plant, hyphae grow within the leaf apoplast, within the xylem elements. Growth within xylem tissues may explain the systemic nature of the fungus within corn plants (Wagner and Lewis, 2000). *B. bassiana* is also well adapted for soil survival as conidia or saprophytic mycelia (Gottwald and Tedders, 1984).

Because of the biology of *B. bassiana* and previous observations of conidia on the surface of corn after a rain event (Lewis, unpublished data), the role of rainfall in the dispersal of conidia from the soil to the surface of corn deserves study. Application of aqueous formulations of *B. bassiana* to soil in long-term tillage regimes showed that movement of conidia through the soil profile was positively correlated with water infiltration rates (Storey and Gardner, 1988). Total vertical movement was restricted and more than 94% of the conidia were recovered from the upper 5 cm of the soil profile (Storey and Gardner, 1988). Filtration of the conidia ( $2\text{--}3\text{ }\mu\text{m} \times 2.0\text{--}2.5\text{ }\mu\text{m}$ ) apparently occurs as they pass through the soil matrix (Storey et al., 1989). Thus, the upper portion of the soil profile would be expected to harbor conidia for transport to the plant surface during a rain event.

Reduced tillage systems have higher inoculum levels of *B. bassiana* than intensely tilled systems (Sosa-Gomez and Moscardi, 1994). Biotic factors suggested to explain this increase in inoculum in reduced tillage systems include soil capacity to retain water, increased organic matter (conservation-till system), and lower

soil temperatures (Vieira, 1981; Bing and Lewis, 1992). Reduction in *B. bassiana* inoculum in intensely tilled systems has been attributed to the incorporation of carbon and nitrogen and the subsequent stimulation of soil microflora (Lingg and Donaldson, 1981). The objectives of these experiments were to study the movement of soil-borne *B. bassiana* to the corn surface during a rain event and determine the impact of crop residue level on fungal transport.

## 2. Experimental procedures

### 2.1. Laboratory studies

Experiments were performed as a randomized complete block design with three replications and four treatments. Treatments were the amount of crop residue covering the surface of the soil in each potting flat. Eight corn plants (Garst 8543, Garst Seed Company, Slater, IA) were grown in  $36\text{ cm} \times 51.5\text{ cm} \times 9.5\text{ cm}$  flats (Dyna Flat, Hummert International, Earth City, MO). Flats were filled with topsoil to within 2 cm of the top. Before placing soil in the flat, the base was lined with a piece of fiberglass screening to allow excess water to drain through the holes in the base. Two rows of four plants were planted in each flat and the appropriate amount of sterilized corn residue applied to the soil surface.

Corn residue used was collected from cornfields harvested mechanically in the fall of 1999 and sterilized at  $220\text{ }^{\circ}\text{C}$ , 20 psi for 20 min in an autoclave. Field-collected residue was used to best simulate the size and condition of residue particles that would occur in the field. The size of the residue ranged 5–15 cm in length to 1.25–5 cm in width. The mean amount of crop residue covering the surface of each residue treatment was 0.53 (control), 34, 59, and 84%. Crop residue on each flat was determined using digital analysis. The residue in the control treatment consisted only of a small amount of plant material and rocks present in the topsoil used, no additional crop residue was added. Pictures of each flat were taken with a digital camera (Olympus Digital Camera, D-600L,  $280 \times 1024$  pixels) from 1.8 m immediately after applying residue. Quantification of percentage crop residue was performed by the Digital Analysis Center, Iowa State University, Ames, IA.

Each flat was treated with 1.5 g of corn kernel granules (14–20 mesh) formulated at  $4.54 \times 10^{14}$  conidia/g of *B. bassiana* Mycotech 726 Mycotech Corporation (now Emerald Bioagricultural Corp., Lansing MI). This rate of granules was selected because it is equivalent to applying 11 lb of granules per acre which is a reasonable rate for the application of a soil insecticide (i.e. Lorsban 15G, Dow AgroSciences, Indianapolis, IN). Granules were spread evenly over the surface of each flat. Flats were watered when the soil surface became dry to the touch by laying a garden hose in the center of each flat and letting the water slowly run and be absorbed without any splash. Flats were placed in a greenhouse at 27 °C, 16:8 L:D photoperiod, until plants reached the V2 growth stage (Richie et al., 1997).

Rain events were simulated at the National Soil Tilth Laboratory, Ames, IA. Once plants reached the two-leaf stage, they were transported to a rain tower calibrated to deliver 73 mm of rain/h. The average volume of the raindrops produced was  $0.148 \text{ cm}^3$ , with a drop surface area of  $1.35 \text{ cm}^2$ . To get a consistent distribution of rain, flats were rained on individually at the same location on the rain platform.

Control plants, which received no rainfall, were cut just above the soil surface and placed into individual sterile bags (Nasco, 18 cm  $\times$  33 cm) after placement of the flat on the rain platform but before rain simulation. Flats were exposed to the above-described intensity of rain for 10 min. Flats were rained on for 10 min to maximize exposure to rainfall while preventing pooling on the surface of the flats. When the rain event was complete, flats were removed from the platform and two plants were randomly selected, cut just above the soil surface, and placed into individual sterile bags.

Fifty milliliters of sterile distilled water were added to each bag. Bags were shaken vigorously for 60 s to dislodge soil and conidia adhered to the surface of the corn plant. One milliliter aliquot of the suspension was placed on each of two plates favoring the growth of *B. bassiana* (Doberski and Tribe, 1980) and placed in a microbial growth chamber at 27 °C, 50% RH. Plates incubated for 10 days were counted for the number of colony forming units (CFU). The count of the two plates for each plant were averaged to give mean CFU per plant. The remaining suspension from each plant was placed into a pre-weighed aluminum pan. Each bag was carefully flushed with distilled

water to remove all soil particles. Pans containing water were then placed in a 38 °C drying oven until dry. The pans were re-weighed and the mg of soil quantified.

Three replications of all treatments were planted and subjected to simulated rainfall on four different occasions (runs). A test of homogeneity of variance was performed to detect variation between runs and treatment  $\times$  run interaction (Little and Hills, 1978). Variability was not significantly different, and the runs were combined to provide a total of 12 replications. Data were analyzed as a split-plot design with rain as the whole-plot and level of crop residue as the split-plot using analysis of variance with the general linear models procedure (GLM) (SAS Institute Inc., 1995). Means were separated using Tukey's multiple range test, with a reference probability of  $P \leq 0.05$  (SAS Institute Inc., 1995). Regression analyses were performed on plants receiving rain to determine the relationships between the mg of soil, number of CFU on the plant surface and the percentage crop residue (SAS Institute Inc., 1995).

## 2.2. Field studies

Field studies were conducted in spring 2000 to validate laboratory findings. Treatments were arranged in a completely randomized design with four replications and two tillage systems: a conservation-till system (two passes with a field cultivator once in the fall and once in the spring, 45% crop residue) and a no-till system (no tillage following the previous years crop, 96% crop residue). All replications consisted of individual 2.5 ha plots that had been in continuous corn and the same tillage regime since the early 1970s. Mean crop residue in each system was quantified by taking eight pictures of each tillage system and having the photos digitally analyzed as described previously.

Hybrid corn (Garst 8543) was planted on 1 May 2000 into half of each plot. The other half was planted with the same hybrid on 17 May 2000. A weather station (CR10; Campbell Scientific Inc., Logan, UT) was placed adjacent to the plots to monitor the intensity and amount of each rain event. Plants were sampled on a weekly basis the first 2 weeks of the study and the day following rainfall  $>10 \text{ mm}$  thereafter. Ten plants were randomly sampled from each plot, placed into

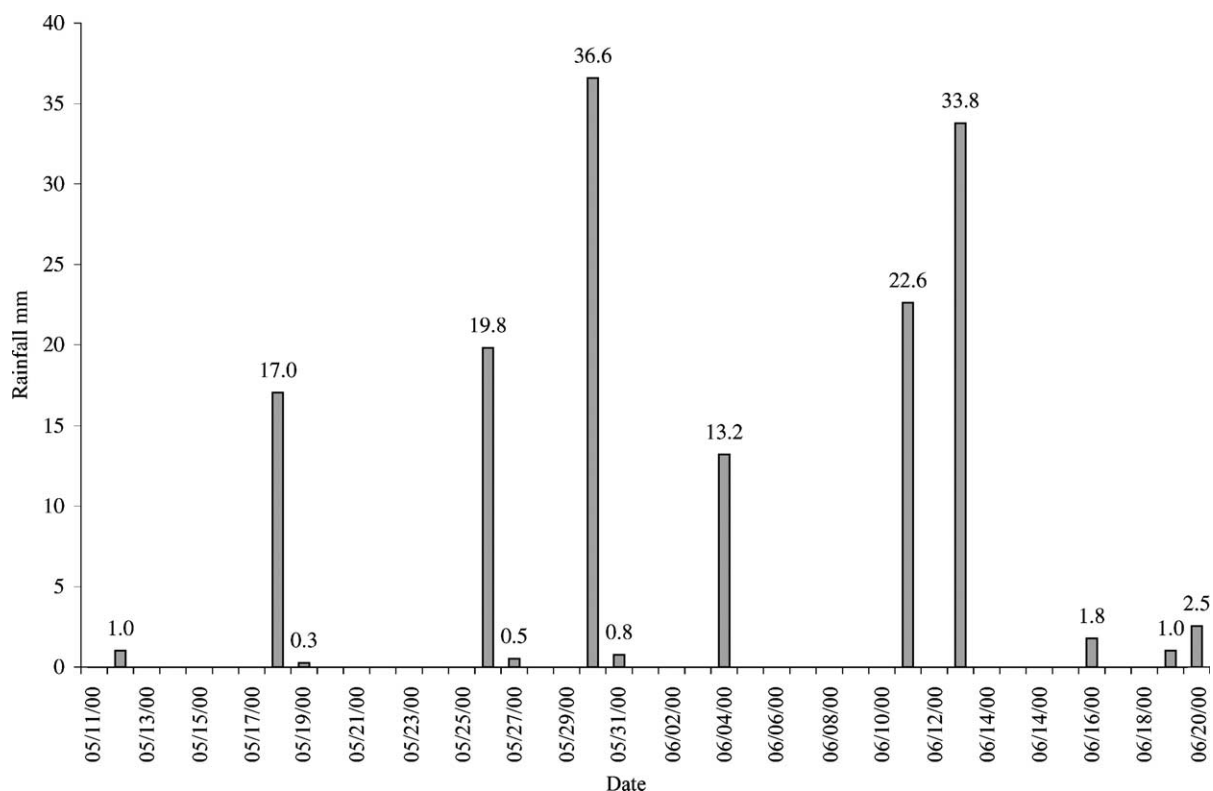


Fig. 1. Daily rainfall (mm) from field validation study to determine the amount of *B. bassiana* and soil splashed onto whorl-stage corn plants.

sterile bags, returned to the laboratory and processed as described previously.

Samples of the first planting began 10 May 2000 when plants were at V1. No rain had fallen on plants since their emergence. The second sampling occurred on 15 May 2000 when the plants were at V2 and also prior to any rainfall. Sample 3 was taken after a rain event when plants were in growth stages V2–V3. Sample 4 was taken 25 May 2000 when plants were V4 prior to the second rain event. Samples 5–8 were taken 27 May; and 1, 12, and 14 June 2000, all after rain events when plants were at V4–V5, V5–V6, V5–V6, and V6, growth stages, respectively. The second planting was sampled beginning 25 May 2000 when plants were at V1, and again no rain had fallen following emergence. The second sample occurred 27 May 2000 when plants were at V1, after a rain event. Samples 3–6 occurred on 1, 12, 14, and 21 June 2000 after rain events when plants were at growth stages V2, V3–V4,

V4, and V5–V6, respectively. Rainfall prior to each sample is presented in Fig. 1.

Because of low fungal transfer initially, *B. bassiana* granules were applied by hand to small areas (1.5 m × 4.5 m) within each plot at the same rate used in the laboratory. Augmentation of field plots took place prior to sample 8 of the first planting and prior to samples 5 and 6 of the second planting.

Data were analyzed separately for each collection date using GLM. Means were separated using Tukey's multiple range tests, with a reference probability of  $P \leq 0.05$  (SAS Institute Inc., 1995). Regression analyses were also performed to determine the relationship between the mg of soil splashed onto the surface of plants and the resulting number of CFU isolated from the surface of those plants (SAS Institute Inc., 1995). Regression analyses were performed on plants after a rain event sampled from areas augmented with *B. bassiana*.

### 3. Results

#### 3.1. Laboratory studies

Laboratory studies demonstrated the ability of rainfall to transfer *B. bassiana* and soil particles to the plant surface. Plants receiving rain had a mean ( $\pm$ S.E.M.) of  $8.8 \pm 2.8$  CFU per plant, whereas controls had a mean of  $0.03 \pm 0.01$  CFU per plant ( $F = 7.05$ ; d.f. = 1, 22;  $P = 0.01$ ). There were no significant differences between crop residue levels in the mean number of CFU per plant ( $F = 0.92$ ; d.f. = 3, 62;  $P = 0.44$ ). There were distinct divisions in the number of CFU per plant between the two low levels of crop residue ( $6.7 \pm 4.5$ ,  $6.7 \pm 3.3$ ) and the two high levels of crop residue, ( $2.5 \pm 1.4$ ,  $2.1 \pm 1.9$ ), respectively. Plants rained on in treatments with the lower amounts of crop residue had almost 2.5 times more CFU per plant than those with the higher residue levels. There were no significant interactions between rain and level of crop residue in the mean number of CFU per plant ( $F = 0.92$ ; d.f. = 3, 62;  $P = 0.44$ ). A regression analysis performed using data from only those plants receiving rainfall showed a significant decrease in CFU per plant as crop residue increased (Fig. 2).

Rainfall also caused a significant increase in the amount of soil transferred to the plant surface. There were significant differences between plants receiving rain and controls in the amount of soil per plant ( $F = 6.86$ ; d.f. = 1, 22;  $P = 0.02$ ). Plants receiving rain had a mean of  $15.7 \pm 1.7$  mg soil per plant, whereas

controls had  $3.4 \pm 0.4$  mg soil per plant. There were also significant differences in the mean amount of soil per plant between crop residue levels ( $F = 7.00$ ; d.f. = 3, 62;  $P = 0.0004$ ). Plants in the 0.53% crop residue treatment had significantly more soil after a rain event than all other treatments. There were no significant interactions between rain and crop residue level in the mean mg of soil per plant ( $F = 1.74$ ; d.f. = 3, 62;  $P = 0.17$ ). A regression analysis performed using data only from those plants receiving rain on the level of crop residue showed a significant decrease in the mg soil per plant as the amount of crop residue increased (Fig. 2).

#### 3.2. Field studies

The field study validated laboratory results. The mean number of CFU and mg of soil collected from each tillage system from the first planting are presented in Table 1. Data of CFU per plant from samples 1–7 represent results from natural field inocula, whereas plants from sample 8 were taken from areas of the field augmented with *B. bassiana*. The mean number of CFU per plant increased substantially when the soil surface was augmented with *B. bassiana*. The mean number of CFU per plant was low in samples 1–7, and there were no significant differences in the mean number of CFU per plant between tillage types. After the soil surface had been augmented with *B. bassiana* there was a significant difference between tillage systems (sample 8). The mean number of CFU per plant was significantly

Table 1

Mean ( $\pm$ S.E.M.) number of CFU and mg of soil collected per plant from corn sampled from the first planting of the field validation study

Sample date	No. of CFU <sup>a</sup>		Soil in mg <sup>a</sup>	
	Conservation-till	No-till	Conservation-till	No-till
10 May	$0.0 \pm 0.0$ a	$0.0 \pm 0.0$ a	$26.0 \pm 19.5$ a	$0.64 \pm 0.3$ b
15 May	$0.01 \pm 0.01$ a	$0.01 \pm 0.01$ a	$5.3 \pm 0.7$ a	$1.0 \pm 0.2$ b
19 May	$0.01 \pm 0.5$ a	$0.02 \pm 0.01$ a	$77.1 \pm 7.2$ a	$7.0 \pm 2.6$ b
25 May	$0.0 \pm 0.0$ a	$0.03 \pm 0.01$ a	$19.8 \pm 2.3$ a	$5.7 \pm 0.8$ b
27 May	$0.4 \pm 0.1$ a	$0.1 \pm 0.05$ a	$134.6 \pm 12.5$ a	$26.8 \pm 8.6$ b
1 June	$3.3 \pm 3.0$ a	$0.8 \pm 0.7$ a	$243.6 \pm 23.1$ a	$25.7 \pm 11.6$ b
12 June	$0.3 \pm 0.1$ a	$0.08 \pm 0.04$ a	$150.9 \pm 10.3$ a	$24.9 \pm 4.2$ b
14 June	$143.1 \pm 16.7$ a	$8.8 \pm 2.8$ b	$322.5 \pm 33.0$ a	$46.5 \pm 12.5$ b

<sup>a</sup> Means under pairs of column headings from the same sample date with different letters are significantly different (Student's *t*-test,  $P \leq 0.05$ ).

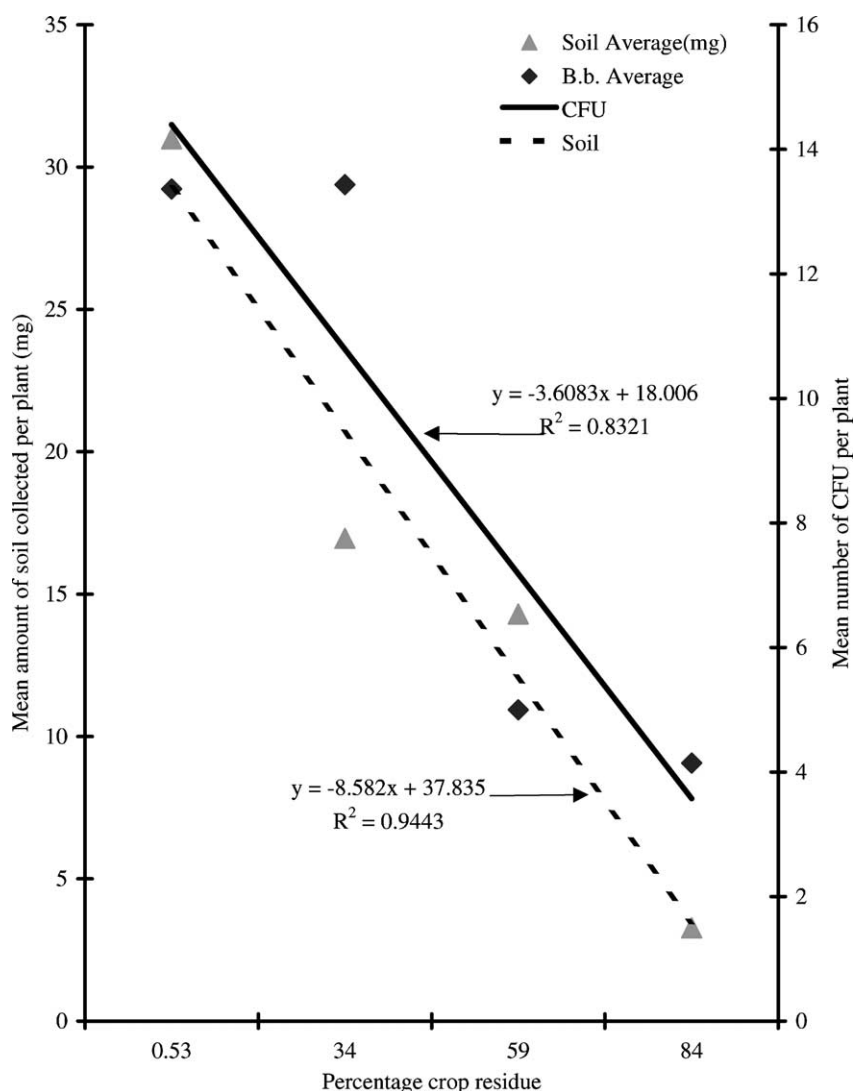


Fig. 2. Linear regression of laboratory results of the mean number of CFU and mg of soil per plant at the four levels of crop residue from plants receiving rainfall.

higher in the conservation-till plots ( $F = 61.11$ ; d.f. = 1, 77;  $P = 0.0001$ ). Results of the number of CFU per plant were similar for plants from the second planting. The number of CFU per plant isolated from plants collected before plots were augmented with fungus (samples 1–4) were low (Table 2). However, after augmentation (samples 5 and 6) the number of CFU per plant was significantly higher in the conservation-till plots than the no-till plots. A regression analysis of the mean number of CFU per plant

on the mean mg of soil per plant from sample 5 and 6 of the second planting accounted for 99% of the variance ( $y = 0.4203x + 6.4258$ ;  $R^2 = 0.99$ ).

Results of soil transfer from both planting dates are similar. Plants collected from the conservation-till plots had significantly more adhering soil than plants from no-till plots on both planting dates. As plant size increased, the amount of soil splashed onto the corn surface also generally increased in both tillage systems.



Table 2

Mean ( $\pm$ S.E.M.) number of CFU and mg of soil collected per plant from corn sampled from the second planting of the field validation study

Sample date	No. of CFU <sup>a</sup>		Soil in mg <sup>a</sup>	
	Conservation-till	No-till	Conservation-till	No-till
25 May	0.06 $\pm$ 0.03 a	0.01 $\pm$ 0.01 a	3.8 $\pm$ 2.5 a	15.5 $\pm$ 8.4 a
27 May	0.0 $\pm$ 0.0 a	0.0 $\pm$ 0.0 a	22.8 $\pm$ 4.0 a	2.6 $\pm$ 0.8 b
1 June	0.7 $\pm$ 0.6 a	0.03 $\pm$ 0.02 a	65.8 $\pm$ 9.7 a	9.6 $\pm$ 5.1 b
12 June	1.3 $\pm$ 1.2 a	0.0 $\pm$ 0.0 a	191.0 $\pm$ 14.4 a	12.9 $\pm$ 1.7 b
14 June	165.7 $\pm$ 21.4 a	14.2 $\pm$ 7.6 b	407.0 $\pm$ 35.6 a	33.0 $\pm$ 4.5 b
21 June	15.5 $\pm$ 6.6 a	1.1 $\pm$ 0.5 b	73.4 $\pm$ 6.4 a	15.1 $\pm$ 1.7 b

<sup>a</sup> Means under pairs of column headings from the same sample date with different letters are significantly different (Student's *t*-test,  $P \leq 0.05$ ).

#### 4. Discussion

In this research we investigated the relationship between rainfall and crop residue in the transfer of the entomopathogenic fungus *B. bassiana* present in the soil to the surface of whorl-stage corn (V1–V6). *B. bassiana* and soil transport to the surface of corn decreased with increasing levels of crop residue. Increased levels of crop residue have also been found to decrease the spread of fungal plant pathogens (Madden, 1997; Reynolds et al., 1987). Transport distance is short, <15 cm in each rain event, indicating that spore deposition on a potential infection site results from several re-splashing events across the soil surface (Madden, 1997). Characteristics of the soil residue dramatically influence conidial movement; increasing surface roughness reduces splash dispersal (Madden, 1997).

Until now, the role of rainfall in the transport of soil-borne entomopathogenic fungi was unknown. These studies demonstrate, that when *B. bassiana* is present on the soil surface, dispersal by rainfall to the surface of corn takes place. The level of crop residue also plays an important role in *B. bassiana* transfer. Naturally occurring *B. bassiana* inoculum is greater in no-till systems, where the mean number of CFU per gram of soil is as much as 44% greater than in tilled systems (Bing and Lewis, 1993; Sosa-Gomez and Moscardi, 1994). However, in no-till systems with increased levels of inocula, the amount of fungal transfer to the plant surface would be limited because of the surface roughness reducing splash dispersal, as well as the lower proportion of soil exposure. The increased inoculum levels seen in no-till systems do not

offset the reduced dispersal of *B. bassiana* to plants. The development of an integrated pest management (IPM) program with *B. bassiana* as an over-the-row soil application at planting, may prove successful in conservation-till systems. The amount of crop residue present in a conservation-till system (Bull and Sandretto, 1996) allows for *B. bassiana* transfer to the plant surface. In addition, the crop residue left on the soil surface in a conservation-till system may also harbor insects infected with *B. bassiana*. As the crop residue decomposes, these infected insects would be exposed and could serve as an additional inoculum source.

Rainfall plays an active role in the epidemiology of *B. bassiana*. During a rain event, soil borne *B. bassiana* is readily transferred to the surface of whorl-stage corn. Whether the levels of fungal transfer obtained are sufficient to infect surface feeding corn pests such as *O. nubilalis*, *Helicoverpa zea* (Lepidoptera: Noctuidae), and adult *Diabrotica* spp. (Coleoptera: Chrysomelidae) is unknown. *B. bassiana* on the plant surface would also be available for endophyte formation (Wagner and Lewis, 2000).

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Iowa Agriculture and Home Economics Experiment Station, Ames: Project 3543. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the Iowa State University or USDA for its use.

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